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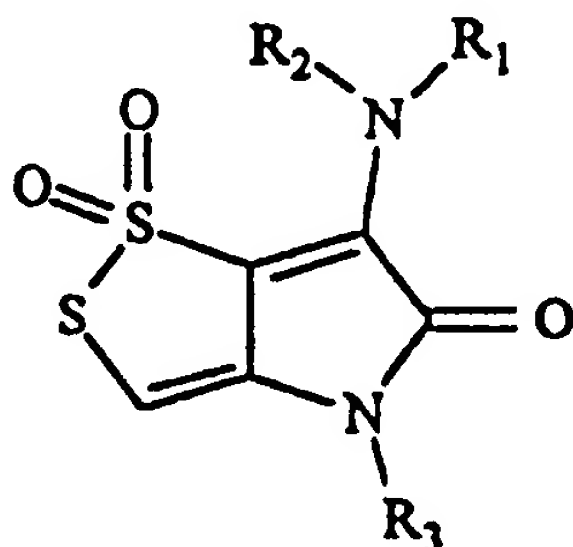
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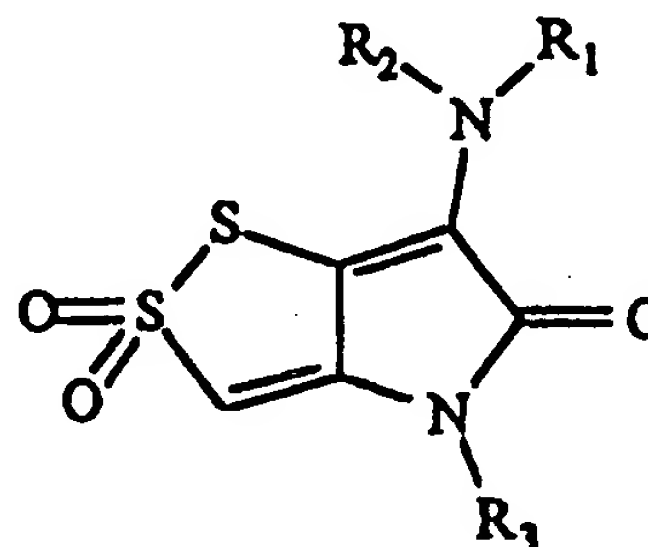
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(54) Title: XENORXIDES WITH ANTIBACTERIAL AND ANTIMYCOTIC PROPERTIES



(A)



(B)

(57) Abstract

The invention is drawn to novel antibiotics, XENORXIDES of formula (A) or (B), wherein R₁, R₂ = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, acyl, aryl, aralkyl, or heterocyclyl group; R₃ = hydrogen, alkyl, cycloalkyl, aralkyl or aryl group, produced by bacterial symbiont *Xenorhabdus bovienii* and/or other *Xenorhabdus* species, and/or by oxidation of the corresponding dithiolopyrrolone derivatives with oxygen and *Xenorhabdus* species, and/or its cell-free filtrate, the additional salts thereof, the compositions thereof and their use as medicaments and/or agrochemicals, particularly in the treatment of infectious diseases involving microorganisms susceptible to them, including drug-resistant *Staphylococcus*.

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XENORXIDES WITH ANTIBACTERIAL AND ANTIMYCOTIC PROPERTIES

DESCRIPTION OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to the novel antibiotics, XENORXIDES, which may be obtained by cultivation of *Xenorhabdus* spp. or by oxidation of the corresponding dithiolopyrrolone derivatives with oxygen and/or *Xenorhabdus* species, and/or its cell-free filtrate.

SUMMARY OF THE INVENTION

The present invention provides the novel antibiotic XENORXIDES having antimicrobial activity. The present invention also provides methods for the production of XENORXIDES, comprising the step of cultivating the microorganism *X. bovienii* or oxidation of the corresponding dithiolopyrrolone derivatives with oxygen and *Xenorhabdus* species, and/or its cell-free filtrate. The present invention further provides novel antimicrobial compositions comprising XENORXIDES, the additional salts thereof, and methods of using the inventive compounds as antibacterial and antimycotic agents.

BRIEF DESCRIPTION OF THE DRAWING

The following Figure 1 represents the structural formula of XENORXIDES, a novel group of compounds,

wherein R_1 , R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, acyl, aryl, aralkyl, or heterocyclyl group; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl or aryl group.

BACKGROUND

Protection of humans, agricultural crops, stored foods, gardens, ornamental plants, trees and wood products, and animals from bacterial and fungal diseases and the sterilizing of medicinal instruments is extremely important. Unfortunately, bacteria and fungi continue to be problematic pathogens for humans because of the increasing occurrence of strains which are resistant to commonly used antibiotics. Such resistant strains lead to a constant need for new, antibacterial and antimycotic substances. As well, the withdrawal of many pesticides from use, for environmental and health reasons, has greatly increased the need for new antifungal and antibacterial agents in the agro-forest industries.

Although there are a limited number of publications on *Xenorhabdus* and *Photorhabdus*, it has been recognized that active, antibacterial and antimycotic substances are produced by *Xenorhabdus* species and *Photorhabdus* species. Some of these specific compounds have been isolated, identified and their structures elucidated (Li *et al.*, "Antimicrobial metabolites from a bacterial symbiont" *J. Nat. Prod.* Vol. 58, 1081-1086 (1995); Paul *et al.*, "Antibiotics in Microbial Ecology: Isolation and Structure Assignment of Several New Antibacterial Compounds from the Insect-Symbiotic Bacteria *Xenorhabdus* spp." *J. Chem. Ecol.* Vol. 7, 589-589-597 (1981); Richardson *et al.*, "Identification of an Anthraquinone Pigment and a Hydroxystilbene Antibiotic from *Xenorhabdus*" *App. Environ.*

Microbiol. Vol. 54, 1602-1605 (1988); McInerney *et al.* "Biologically Active Metabolites from *Xenorhabdus* spp., Part 1. Dithiolopyrrolone Derivatives with Antibiotic Activity" *J. Nat. Prod. Vol. 54*, 774-784 (1991a); McInerney *et al.* "Biologically Active Metabolites from *Xenorhabdus* spp., Part 2. Benzopyran-1-one Derivatives with Gastroprotective Activity" *J. Nat. Prod. Vol. 54*, 785-795 (1991b)). Recently, the cell-free culture broths of *Xenorhabdus* species and *Photorhabdus luminescens*, bacterial symbionts carried by nematodes of the genus *Steinernema* and *Heterorhabditis*, respectively were found to be active against many fungi of agricultural and medicinal importance (Chen *et al.*, Antimycotic activity of two *Xenorhabdus* species and *P. luminescens*, bacteria associated with the nematodes *Steinernema* species and *Heterorhabditis megidis*. *Biological Control. Vol. 4*, 157-162(1994)). However, XENORXIDES, as a novel group of chemicals and the importance of these specific purified metabolites as extremely potent antibacterial and antimycotic agents has heretofore been undiscovered, and are the subjects of this invention. Although corresponding dithiolopyrrolone derivatives have been shown to be active against microorganisms, prior art references have not shown the existence of XENORXIDES and the use of XENORXIDES or any operable aspects as antibacterial and antimycotic agents.

DESCRIPTION OF THE INVENTION

The microorganisms

Xenorhabdus bovienii and its nematode symbiont *Steinernema feltiae* used in this study were collected from soil in British Columbia, Canada and maintained in culture in Dr. J. M. Webster's laboratory in the Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6 (Maxwell *et al.* 1994). Briefly, last instar larvae of the greater wax moth, *Galleria mellonella*, were infected with infective juvenile (IJ) nematodes, carrying the *X. bovienii* A21 strain, at a rate of 25 IJs/larvae. After 24 to 48 h the dead insect larvae were surface disinfected by dipping them into 95% EtOH and igniting them. The cadavers were aseptically dissected, haemolymph was streaked onto an agar culture medium and incubated in the dark at room temperature. The agar medium has the following composition in one litre of distilled water:

beef extract	3 g
peptone	5 g
bromothymol blue	0.025 g
2,3,5-triphenyltetrazolium	0.04 g
Agar	15 g

Sterilized at 121 °C for 15 minutes.

The resulting primary form of *X. bovienii* was maintained and subcultured at 14 d intervals. Other sources and depositories of *Xenorhabdus* species and strains are noted in Akhurst and Boemare "A numerical taxonomic study of the genus *Xenorhabdus* (Enterobacteriaceae) and proposed elevation of the subspecies of *X. nematophilus* to species" *J. Gen. Microbiol.* Vol 134, pp.1835-1845 (1988). Putz *et al.* "Development and application of oligonucleotide probes for molecular identification of *Xenorhabdus* species" *Appl. Environ. Microbiol.* Vol. 56, 181-186 (1990) notes additional sources and depositories, including the American Type Culture Collection, Rockville, MD. Candidate bacterial and fungal pathogens used in bioassays are readily available from many sources, including the American Type Culture Collection, Rockville, MD. For consistency, 14% sucrose lyophilized powder of bacteria stored at -20°C was frequently used as starting material for cultures.

Cultures of *X. bovienii* A21 strain which have been obtained as above exhibit the following characteristics:

Gram reaction	-*
Cell size (µm)	5.3×2.2
Mobility	+
Cell peritrichous	+
Pigmentation	yellow
Catalase	-
Oxidase	-
Urease	-
Lecithinase	+
Lipase(Tween 80)	+
Acid production	+w'
D-Arabinose	-
Esculine	+
D-Fructose	-
D-Galactose	+
D-Glucose	+w
Inositol	-
Inulin	-
D-Lactose	+
D-Maltose	-
D-Mannitol	+
D-Mannose	-
D-Raffinose	+w
D-Sorbitol	-
L-Sorbose	-
D-xylose	-
Utilization of carbon sources	+
Asparagine	-
Cystine	-
Glycine	+
Tyrosine	-
Nictinic acid	-

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* + positive; - negative; +w: weakly positive.

These characteristics are in agreement with those described for *Xenorhabdus bovienii* by Akhurst, R. J. and N. E. Boemare, *J. Gen. Microbiol.* Vol. 134, 1835-1845 (1988), and, therefore, establishes the identity of the organism as *Xenorhabdus bovienii*.

Production of XENORXIDES

Cultivation of the microorganism *X. bovienii* yields the novel antimicrobial substances, XENORXIDES. XENORXIDES may be formed as metabolites thereof.

To prepare XENORXIDES, *X. bovienii* may be cultivated (fermented), for example, at about 25 °C under submerged aerobic conditions in an aqueous nutrient medium containing assimilable carbon (carbohydrate) and nitrogen sources until antibiotic activity due to XENORXIDES is imparted to the medium. The fermentation may be carried out for a time period such as approximately 48 to 96 hours, at the end of which time the antibiotic XENORXIDES have been formed, and may be isolated from the fermentation medium and purified.

After the fermentation has been completed, the fermented broth may be filtered or centrifuged and the pH of the filtrate adjusted to about 7.0 by the addition of hydrochloric acid or kept as it was. The filtrate may then be extracted with a water immiscible organic solvent, for example, with ethyl acetate or chloroform. The combined organic layers (e.g. pooled ethyl acetate or chloroform extracts) may be concentrated in vacuum (e.g. at about 30 °C.) to an oily residue ("syrup"). The oil may be mixed with a small amount of organic solvent and chromatographed on a silica gel column. After introduction of the sample, chloroform or other organic solvent may be applied to elute the bioactive fraction out. The bioactive fraction may be purified further by high resolution liquid chromatography (HPLC) with organic and/or aqueous solution.

XENORXIDES are difficult to detect in the culture broth of *X. bovienii*, but the corresponding dithiolopyrrolone derivatives are present in relatively large amounts. Therefore, the culture broth of *X. bovienii*, with corresponding dithiolopyrrolone derivatives present in relatively large amounts, may be filtered or centrifuged. The cell-free filtrate may be open to the air for extended periods from one week up to one month with or without stirring at room temperature or other temperature. This process may oxidize all or part of the corresponding dithiolopyrrolone derivatives to XENORXIDES, thus providing a practical way to produce XENORXIDES.

The Antibiotic and Use Thereof

XENORXIDES possess antibacterial and antimycotic properties, and have been found to have the characteristics shown in the Figure 1 and in the Examples herein.

The compounds of the present invention include XENORXIDES and the additional salts thereof. It is preferred that the inventive compounds have a degree of purity such that they are suitable for use as antibiotic agents. A particularly preferred embodiment of the instant invention provides XENORXIDES in a substantially pure state. The substantially pure compounds are preferably employed in the compositions and methods described following.

The inventive compounds are useful as antimicrobial agents, useful in inhibiting the growth of microorganisms, particularly as an antibiotic drug, useful in treating bacterial infection caused by antibiotic resistant bacteria such as Gram positive bacteria, e.g. bacteria of the genera *Bacillus* and *Staphylococcus*, useful in treating infection caused by fungi and yeasts of the genera *Aspergillus*, *Botrytis* and *Cryptococcus*. Inhibition of the growth of a bacterium or fungus may be achieved by contacting the organism with a compound of the present invention in an amount effective there.

Thus, the compounds of the present invention may be employed in utilities suitable for antibacterial and antimycotic agents.

The inventive compounds may, for example, be used in treating a host infected with a bacterium and/or fungus, comprising the step of administering to the host XENORXIDE(S) or a physiologically tolerated salt thereof in an amount effective for the treatment. Treatment of such infections according to the instant invention includes both mitigation as well as elimination thereof.

Hosts treatable according to the method of the present invention include plants and animals, particularly mammals such as dogs, cats and other domestic animals and, especially, humans. The dosage form and mode of administration, as well as the dosage amount, may be selected by the skilled artisan. The dosage amount will vary with the severity of the infection, and with the size and species of the host. Exemplary daily dosages for an adult human are those within the range of about 2.5 mg to about 2,000 mg/day. Administration to a mammalian host may, for

example, be oral, parenteral, or topical. Administration to a plant host may be accomplished, for example, by application to seed, foliage or other plant part, or to the soil.

Compositions are also provided by the present invention which comprise XENORXIDE(S) and/or the additional salts thereof in an amount effective for the treatment of infection by a microorganism, and a physiologically tolerated vehicle or diluent. The term "physiologically tolerated" is equivalent to the term "pharmaceutically acceptable" when used in reference to the treatment of a mammalian host. The appropriate solid or liquid vehicle or diluent may be selected, and the compositions prepared, by methods known to the skilled artisan. Treatment of simultaneous infections by more than one bacterium and/or fungus is, of course, contemplated.

The inventive compounds may be employed also as antibacterial and antimycotic agents useful in inhibiting the growth of microorganisms present or eradicating microorganisms on a surface or in a medium outside a living host. The present invention, therefore, provides a method for inhibiting the growth of at least one microorganism present on a surface or in a medium, comprising the step of contacting the surface or medium with XENORXIDE(S) in an amount effective for the inhibition. Thus, the inventive compounds may be employed, for example, as disinfectants for a variety of solid and liquid media susceptible to microbial growth. Suitable amounts of the inventive compounds may be determined by methods known to the skilled artisan. Compositions comprising XENORXIDE(S) in an amount effective for inhibiting the growth of at least one bacterium, and a vehicle or diluent, are also provided by the present invention.

For agricultural application, the bactericidal and fungicidal compositions may be formed using one of the active ingredients in an inert carrier. If formulated as a solid, the ingredients may be mixed with typical carriers such as Fuller's earth, kaolin clays, silicas or other wettable inorganic diluents. Free-flowing dust formulations may also be utilized by combining the dry active ingredient with finely divided solids such as talc, kieselguhr, pyrophyllite, clays, diatomaceous earth and the like.

The powders may also be applied as a suspension or solution, depending on the solubility in the liquid carrier. Pressurized sprays, typically aerosols with the active ingredient dispersed in a low-boiling dispersant solvent carrier, may be used. Percentages of weight may vary according to the manner in which the composition is to be applied, and formulation used.

In general, the active ingredient will comprise 0.005% to 95% of the active ingredient by weight in the bactericidal and fungicidal composition. The bactericidal and fungicidal composition may be applied with other ingredients, including growth regulators, insecticides, fertilizers, and the like. Formulation of the active ingredients to assist applicability, ease handling, maintain chemical stability and increase effectiveness may require addition of various materials. Solvents may be chosen on the basis of affecting the solubility of the active ingredient, fire hazard and flash point, emulsifiability, specific gravity and economic considerations. Adjuvants may be added to enhance the active ingredients, and can include surfactants which are anionic, cationic or nonionic. Stabilizers and antifreeze compounds will prolong storage. Additionally, synergists, stickers, spreaders and deodorant compounds can be added to improve the handling characteristics of the commercial formulation. Alternatively, the active ingredient can be combined with an inert carrier, such as calcium carbonate, and formed into a pill or other consumable delivery device, including controlled release devices intended to deliver metered doses of the active ingredient.

The following examples are provided to further illustrate the invention, and are not intended to in any way limit the scope of the instant claims.

EXAMPLE 1.

Preparation of XENORXIDES

A. Isolation of XENORXIDES from the cultural broth of *X. bovienii*

Cultures were shaken at 180 rpm on an Eberbach gyrorotary shaker for 24 h at 25 °C. Bacterial fermentation was initiated by adding 100 ml of this bacterial culture to 900 ml of tryptic soy broth in a 2,000 ml flask. The flask was incubated in the dark at 25 °C on a gyrorotary shaker. After 96 h, the culture was immediately centrifuged (12,000 g, 20 minutes, 4 °C) to separate the bacterial cells. The cell-free broth was then extracted with ethyl acetate 4 times. The combined extracts were dried with anhydrous sodium sulfate and then filtered through filter paper. The filtrate was concentrated on a rotary evaporator below 30 °C under vacuum to yield a brown oil. After the above experiment was repeated 10 times, approximately 3 g of the oil was obtained. The crude extracts were then loaded onto a silica

gel (200g silica gel 60, 40 cm x 5 cm, EM Science, Darmstadt, Germany) chromatographic column. The yellow bioactive fraction was eluted out with ether or ethyl acetate. This bioactive fraction was then subject to HPLC on a C18 preparative column (Spherisorb 10 (ODS(1)), 250 X 10 mm, 10 micro, Phenomenex, Torrance, CA) with a program (isocratic at 10% acetonitrile in water for 5 min, then gradually increasing to 85% acetonitrile in 35 min, isocratic for 5 min, then decreasing back to 10% in 2 min) at 2.5 ml/min. The eluate was monitored at 254 nm. XENORXIDE 1 (about 0.3 mg per liter of the cultural broth) was eluted at 33.6 min, and XENORXIDE 2 (0.2 mg/l) was eluted at 35.2 min.

B. Preparation of XENORXIDES from *X. bovienii*

The cell-free broth was obtained using the same method as described above, and was then stored at 4 °C to room temperature for 3 to 6 weeks to allow the corresponding dithiolopyrrolone derivatives produced by the bacterium to be oxidized into xenorxides. Then the aqueous broth was extracted with ethyl acetate, and the combined extracts were separated using the same process discussed above. XENORXIDE 1 was eluted at 33.6 min (2 mg/l), XENORXIDE 2 was eluted at 35.2 min (1.5 mg/l).

C. Identification of the active components from *X. bovienii*

NMR spectra were recorded on a Bruker WM400 spectrometer in CDCl₃, using residual CDCl₃ (~7.25) as internal standard. Low resolution mass spectra were obtained on a Hewlett-Packard 5985B GC/MS system operating at 70 eV using a direct probe. High resolution MS spectra were recorded on a Kratos MS80 instrument. IR spectra were recorded as neat film on NaCl using a Perkin-Elmer 599B spectrometer. (Abbreviations used as follows: EI = Electron Impact, M⁺ = Molecular Ion, t = triplet, J = coupling constant, Hz = Hertz, d = doublet, m = multiplet, bs = broad singlet).

XENORXIDE 1: EIMS: 317(2), 316(M⁺, 13), 220(9), 219(9), 218(100), 186(23), 154(16), 99(40), 71(39); HRMS: 316.0555 (Calc. for C₁₂H₁₆N₂O₄S₂: 316.0551, 20), 217.9824 (Calc. for C₆H₆N₂O₃S₂: 217.9820, 100), 154.0197 (Calc. for C₆H₆N₂OS: 154.0201, 16); IR (KBr): 3448, 3298, 3275, 1720, 1686, 1654, 1637, 1560, 1522, 1310, 1139, 551 cm⁻¹; ¹H NMR (CDCl₃) δ: 7.56 (1H, bs, CO-NH), 6.35 (1H, s, H-3), 3.20 (3H, s, N-Me), 2.38 (2H, t, CO-CH₂, J=7.4 Hz), 1.67 (2H, m, CH₂), 1.32 (4H, m, CH₂CH₂), 0.89 (3H, t, J=7.0 Hz); ¹³C NMR

(CDCl₃) δ : 171.6(s, CON), 164.7 (s, CO), 145.4(s, C7), 121.3(s, C₆), 116.2(s, C₈), 109.2(d, C₃), 36.4, 31.2, 27.8, 24.6, 22.3, 13.8.

XENORXIDE 2: EIMS: 330(M⁺, 10), 218(100); HRMS: 330.0707 (Calc. for C₁₃H₁₈N₂O₄S₂: 330.0708, 18), 217.9829 (Calc. for C₆H₆N₂O₃S₂: 217.9820, 100), 154.0213 (Calc. for C₆H₆N₂OS: 154.0201, 16); IR (KBr): 3438, 3298, 1719, 1686, 1654, 1637, 1560, 1522, 1400, 1310, 1142, 551 cm⁻¹; ¹HNMR (CDCl₃) δ : 7.56 (1H, bs, CO-NH), 6.35 (1H, s, H-3), 3.20 (3H, s, N-Me), 2.36 (2H, t, CO-CH₂, J=7.4 Hz), 1.67 (2H, m, CH₂), 1.2-1.6 (1H, m, CH), 1.22 (2H, m, CH₂), 0.89 (6H, d, J=6.6Hz); Different NOE experiment showed the NOE effect between the peak at 6.35 ppm and 3.20 ppm; ¹³CNMR (CDCl₃) δ : 171.6(s, CON), 164.7 (s, CO), 145.4(s, C7), 121.3(s, C₆), 116.2(s, C₈), 109.2(d, C₃), 38.2(t, CH₂), 36.7(t, CH₂), 28.0(q, CH₃), 27.8(d, CH), 22.8(t, CH₂), 22.4(q, CH₃).

EXAMPLE 2.

XENORXIDES as antibiotic

The following experiments were conducted, demonstrating the antibiotic properties of XENORXIDES.

To determine minimum inhibitory concentration (MIC) of the XENORXIDES, the standard procedures (The National Committee for Clinical Laboratory Standards and Methods for Evaluating Pesticides for Control of Plant Pathogens of the American Phytopathological Society) for testing antibiotics was followed. Briefly, test chemicals were dissolved in dimethyl sulphoxide (DMSO), filter sterilized and diluted into with distilled water resulting in a final DMSO concentration < 0.4%(v/v) at a chemical stock concentration of 200 μ g/ml. The active compounds were serially diluted by twofold (or mixed with equal amount of media /agar) to produce culture media containing the compound from 100 μ g/ml to 0.1 μ g/ml (i.e. 100, 50, 25, 12.5, 6.3, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1) for the determination of MICs. Test bacteria and the test yeast (*Cryptococcus neoformans*) were grown on nutrient agar(potato dextrose agar for the yeast) for 24h (35C), then were scraped from the plate by flooding the plate with 0.8% saline and diluted with the saline to make inocula (containing 2.5-2.8x10⁷ cells/ml). *Aspergillus* spp. and *Botrytis cinerea* were grown

on potato dextrose agar for 7d (25°C) before the conidia were harvested by flooding the plate with sterile, distilled water and diluted to make the final inocula ($2.5-3.0 \times 10^6$ conidia/ml). The inoculated test media were incubated at 35 °C (*B. cinerea* 24 °C) and the MICs were visually determined after 24h incubation (2d for *B. cinerea*). The minimum inhibitory concentration is defined as the lowest chemical concentration which prevents the growth of the test organism at the above conditions.

It was found that similar results were obtained from both liquid and agar culture methods. Table 1 shows the MICs determined for the compounds against each microorganism. In conclusion, it is shown that XENORXIDES, isolated from *Xenorhabdus* have potent antimicrobial properties, in particularly against some antibiotic resistant *Staphylococcus* strains.

TABLE 1: Minimum Inhibitory Concentrations(MIC) of antibiotics isolated from *Xenorhabdus* species on bacteria and fungi.

Organisms	MICs(μg/ml)	
	XENORXIDE1	XENORXIDE2
<i>Bacillus subtilis</i>	6	6
<i>Micrococcus luteus</i>	25	6
<i>Staphylococcus aureus</i> ATCC 29213	6	6
<i>S. aureus</i> 0012*	3	3
<i>S. aureus</i> 0017*	3	1.5
<i>Aspergillus fumigatus</i> ATCC 13073	0.75	1.5
<i>Aspergillus flavus</i> ATCC 24133	0.75	1.5
<i>Botrytis cinerea</i>	12	25
<i>Cryptococcus neoformans</i> ATCC 1411	6	6

*clinical isolates of multi-antibiotic-resistant isolates, provided by S. Farmer of the Canadian Bacterial Diseases Network, Vancouver, British Columbia, Canada.

While our above description contains many specificities, these should not be construed as limitations on the scope of the invention, but rather as examples of preferred embodiments.

Accordingly, the scope of the invention should not be determined by the embodiments presented, but by the appended claims and their legal equivalents.

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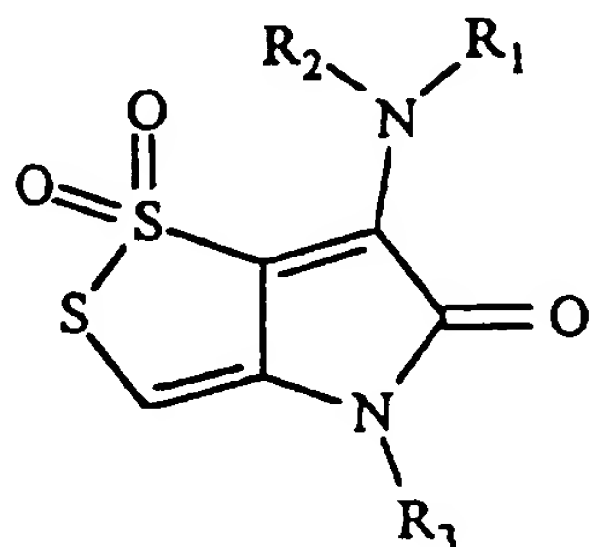
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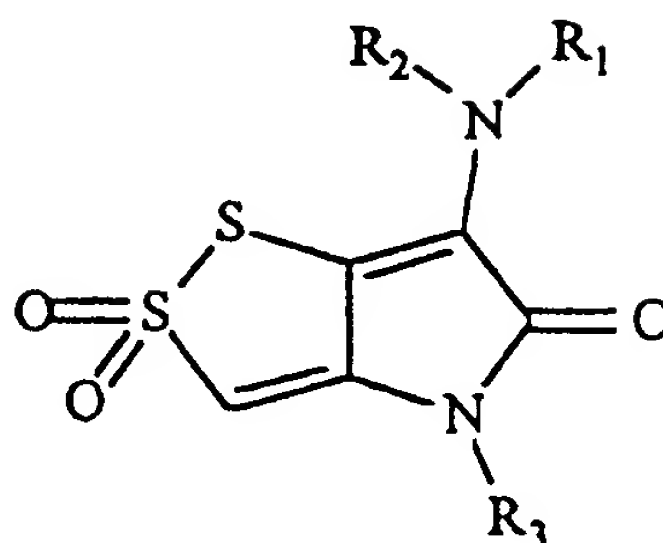
CLAIMS

What is claimed is:

1. A compound of the structure shown below,



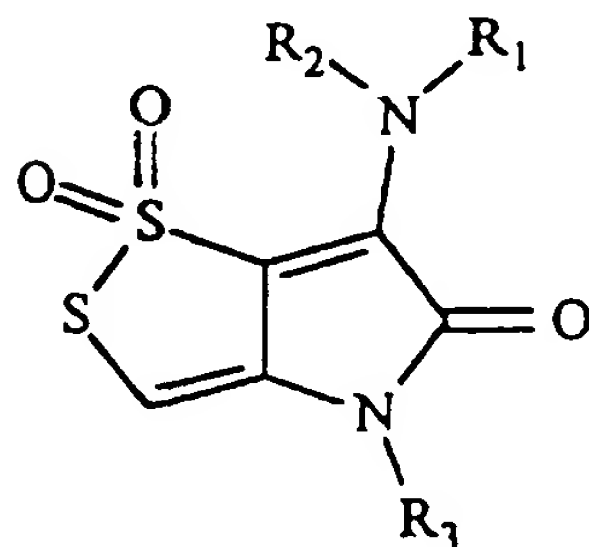
A



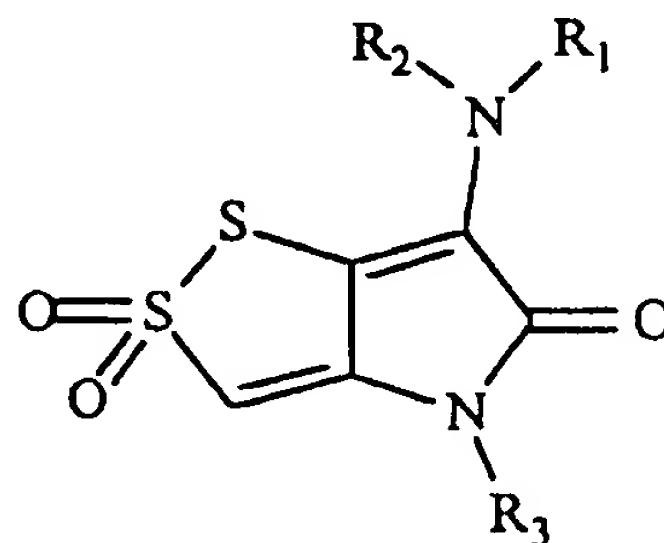
B

wherein R_1 , R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, acyl, aryl, aralkyl, or heterocyclyl group; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl or aryl group, a salt thereof.

2. A compound of claim 1 wherein R_1 = hydrogen, alkyl group; R_2 = acyl group; and R_3 = hydrogen, CH_3 , or other alkyl group.
3. A compound of claim 1 wherein R_1 =hydrogen; R_2 = acyl group with a straight or branched one to ten carbon chain; and R_3 =hydrogen or CH_3 .
4. The process for the production of compound(s) of the structure shown below,



A



B

wherein R_1 , R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, acyl, aryl, aralkyl, or heterocyclyl group; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl or aryl group, by

bacterial fermentation with *Xenorhabdus* or by oxidizing the corresponding dithiolopyrrolone derivative(s) with oxygen and/or *Xenorhabdus* species.

5. A composition for combating bacteria and/or fungi, comprising a carrier and an antifungally and/or antibacterially effective amount of a compound of claims 1, 2 and 3.
6. A pharmaceutical composition of Claims 1,2 and 3 wherein said effective amount is sufficient to treat infections caused by bacteria and fungi on animals and humans.
7. A composition of Claims 1,2 and 3 wherein said effective amount is sufficient to kill and/or inhibit bacteria and fungi on desirable agricultural crops and botanical species.
8. A composition of Claims 1, 2 and 3 wherein said effective amount is sufficient to kill and/or inhibit bacteria and fungi present on a surface or in a medium outside a living host.

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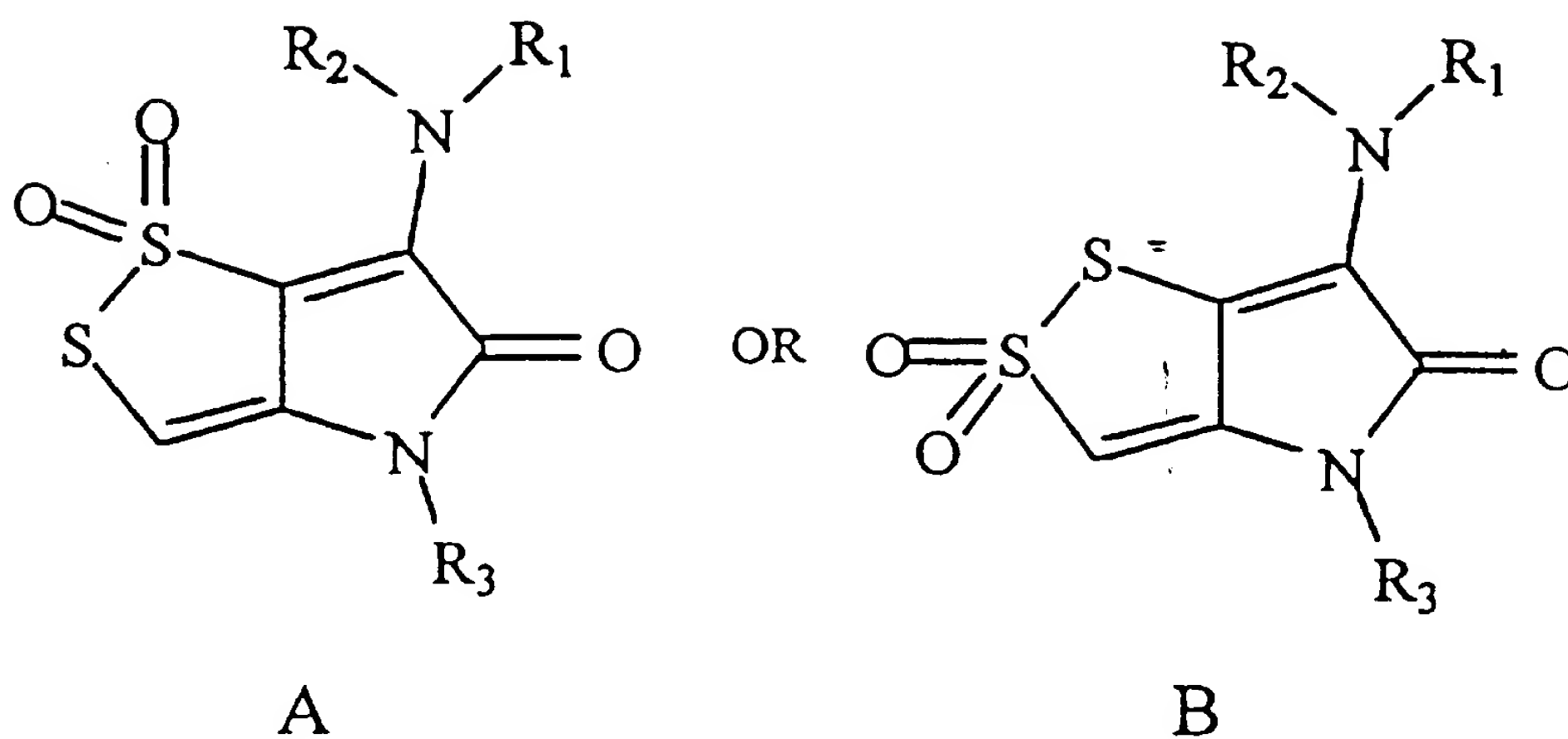


Figure 1

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

Int. Application No.

PCT/CA 96/00217

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D495/04 C12P17/18 A61K31/40 //(C07D495/04,339:00,
209:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D C12P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,84 01775 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 10 May 1984 see page 2, line 16 - line 17; claim 1 ---	1,5
A	EP,A,0 595 458 (SANKYO) 4 May 1994 see page 5, formulas B1 and B2 and claim 16 -----	1,5



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

31 July 1996

Date of mailing of the international search report

7.08.96

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Authorized officer

Alfaro Faus, I

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 96/00217

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		US-A- 4672130	09-06-87

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		HU-A- 70185	28-09-95
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		NO-A- 933328	21-03-94
		NZ-A- 248696	28-03-95
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		ZA-A- 9306840	14-04-94
		JP-A- 6199865	19-07-94
		JP-A- 6206884	26-07-94
		JP-B- 7094458	11-10-95
